INVITRO ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS BY TURBIDITY METHOD

Thesis submitted to

The Tamilnadu Dr. M.G.R Medical University, Chennai

In partial fulfillment of the requirements

For the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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SULUR, COIMBATORE

SEPTEMBER-2009

ACKNOWLEDGEMENT

I offer flowers of gratitude to the almighty God & to my Parents who have been the source of strength throughout my life.

I express my warmest gratitude to our beloved Principal **Dr. R. Venkatnarayanan**, There constructive criticism, perpetual encouragement, timely advice and meticulous attention were the real driving forces as well as his keen interest in my project encouraged me a lot.

I wish my Keep sense of gratitude and sincere thanks to Guide **Mr.R.Suresh,** M.Pharm, Dept. of Pharmacology, RVS College of Pharmaceutical Sciences Sulur, Coimbatore (TN), under whose active guidance, innovative ideas, constant inspiration, encouragement valuable suggestions, the work was entitled as

"IN VITRO ANTI-MICROBIAL ACTIVITY OF PLANT EXTRACTS BY TURBIDITY METHOD"

My special thanks to my coordinator **Dr. B.N Shringi ,**College of Veterinary & Animal Science, Rajasthan Agricultural University, Bikaner for his valuable guidance, inspiration and also for providing the excellent facilities for my work. It is hard to put in words, in its real sense, the care and affection given by him for my work.

I express my sincere and heartily thanks to **Dr.Benito Johnson,** Pharm Ph.D, and Head of dept. of Pharmacology, RVS College of Pharmaceutical Science Sulur, Coimbatore (TN) for his scholastic guidance, moral support, and persistent motivation entire period of course.

I am deeply indebted to **A.K. Gahlot**, Dean. Rajasthan Agricultural University, Bikaner who established this reputed organization and provided opportunity to the students like me to work with such a prestigious Department.

It gives me immense pleasure in expressing my sincere regards and gratitude to **Dr. S.K. Kashyap,** Head Department of Veterinary Microbiology & Biotechnology who provided me this opportunity to work with such a prestigious organization.

I extend my thanks to **Miss. C.Maheswari ,**lecturer Pharmacology for their valuable guidance for my dissertation work.

I sincerely acknowledge **Mr. Sakthivel**, Lab Technician for their constant valuable suggestions during my work.

I am hearty thankful to all my batch mates especially, ***Mr.Irfan, Mr.Manoj, Mr.Narendra, Mr. Samba, Mr.NVSSKishor, Mr.Chandrashekhar, Mr.Kishor, Mr.Vivek***

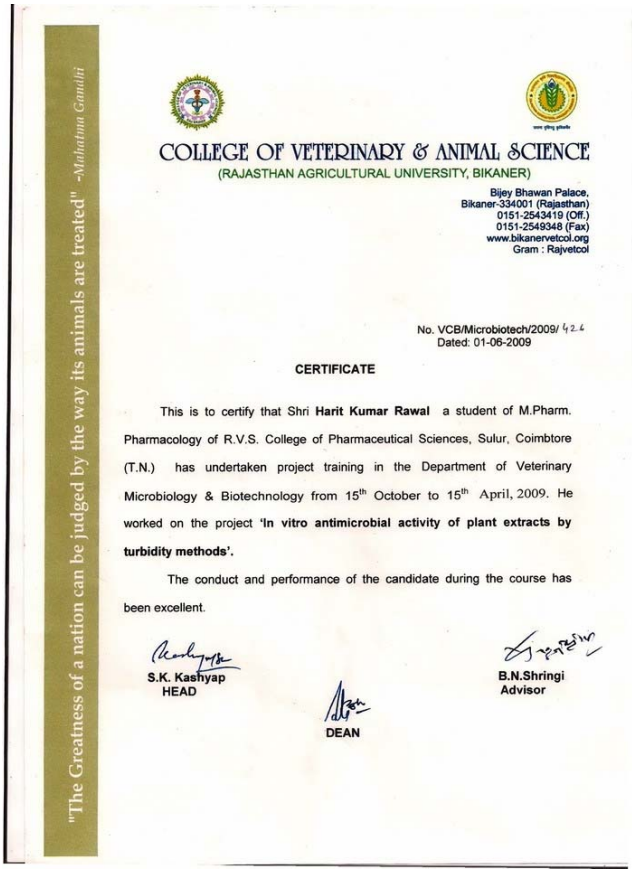
for giving me moral support and help during my dissertation work.

I am hearty thankful to my batch mate **Mr. Dinesh Patidar** for his timely help in Cooperation Whenever I needed.

It will be my Pleasant duty to express my infinite thanks to **Mrs. Varnika H.Rawal** for her timely help and cooperation.

Last, but not least, I express my gratitude and apologize to everybody whose contributions, I could not mention in this page.
With This I Remain

HARIT KUMAR RAWAL



"The Greatness of a nation can be judged by the way its animals are treated" -Mahatma Gandhi

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CERTIFICATE

This is to certify that Shri **Harit Kumar Rawal** a student of M.Pharm. Pharmacology of R.V.S. College of Pharmaceutical Sciences, Sulur, Coimbtore (T.N.) has undertaken project training in the Department of Veterinary Microbiology & Biotechnology from 15th October to 15th April, 2009. He worked on the project **'In vitro antimicrobial activity of plant extracts by turbidity methods'.**

The conduct and performance of the candidate during the course has been excellent.

S.K. Kashyap
HEAD

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DEAN

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INTRODUCTION

Introduction

Microbiology is emerging as the key biological science. Microorganisms provide the models used in molecular biology for research. This research at the molecular level has provided, and continues to provide, the answers to numerous fundamental questions in genetics, metabolism, and cell forms and functions. Microorganisms also provide model systems for studying the relationships between species in mixed populations.

The course of an infection is determined by three interacting factors: the microorganism, host resistance and treatment. The most important of these is the interaction between the host and the pathogenic microorganisms, i.e. the balance between the virulence of the pathogens and the resistance of the host to the pathogens. The role of antimicrobial agents, although often decisive, is mainly to shift the balance in favor of the host, giving the host time to metabolize its resistance mechanisms. Some bacterial species are naturally resistant to certain classes of antibiotics, either because they lack the necessary receptor or because their cell wall is impenetrable to the drug. There are several ways in which bacteria may acquire resistance. The most common mechanism of resistance is that the microorganisms acquire an enzyme that destroys the antibiotics. An important factor in the spread of resistance is the transfer of genetic material from one microorganism to another, even from a non-pathogen to a pathogen. Many pathogenic bacteria have developed resistance to the commonly used antibiotics.

Antibiotics resistant in the bacteria spread at three levels:

I. By transfer of bacteria between people.

II. By transfer of resistant genes between bacteria (usually on plasmids).

III. By transfer of resistant genes between genetic elements within the bacteria, on transposons.

Tranposons: Some stretched of DNA can be fairly readily transferred (transposed) from one plasmid to another and also from plasmid to chromosomes or vice versa. This is because integration of these segments of DNA, which are called transposons.

Antimicrobial drugs have greatest contribution to therapeutics. They are one of the few curative drugs. Antibiotics are the substances produced by microorganisms, which suppress the growth of or kill other microorganisms at very low concentrations. An antibiotic is said to have a narrow spectrum of activity, if it is effective against either Gram-positive or Gram-negative bacteria. Antimicrobial drugs can be classified in many ways according to their chemical structure, mechanism of action, types of organisms, spectrum of activity, type of action, source of origin. Antibiotics are bactericidal or bactriostatic. Bacteriostatic antibiotics inhibit the growth and multiplication of bacterial cell, without killing them, but allow host factors to eliminate the pathogens. Bactericidal antibiotics kill and sometimes lyse the cells. According to their nature and dose concentration they are given. Bactericidal drugs are Penicillin, Cephalosporin, Cephamycin, Aminoglycocides, Glycopeptides, Polymixin, Bacitracin, Monobactams, Carbapenems and bacteriostatic drugs are Tetracycline, Chlormpenicol, Clindamycin, Sulphonamides, Trimethoprim, and Macrolides.

Sensitivity testing for antibiotics is based mainly on quantitative criteria, such as the minimum inhibitory concentration (MIC). The MIC is the minimum concentration that prevents visible growth of standard inoculum of bacteria after 18-24 hours incubation.

Plants are known to possess several antimicrobial compounds and are used in all traditional medicine. Many crude preparations of "herbal drugs'' are in clinical use in medical and veterinary practice. Scientists from divergent fields are investigating plants a new with an eye to their antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganism's invitro. More of these compounds are being subjected to animal and human studies to determine their potential to restrict growth / multiplication of pathogenic organisms as well as examination of their effects on beneficial normal micro biotech. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent year.

While 25-50 % of current pharmaceuticals derived from plants none are used as antimicrobials. Traditional healers have long used plants to prevent or cure the infectious condition. Plants are rich in a wide variety of secondary metabolite such as tannins, terpenoids, alkaloids, and flavenoids which have been found invitro to have antimicrobial properties.

Major part of the Rajasthan state covers unique ecosystem i.e. Thar Desert that is rich in several unique plants and shrubs advocated to this climate. Traditional healers are known to employ many applications in the treatment of infectious and none infectious diseases which are derived from locally available medicinal plants. The description about such plants and medicines are available in local literature it thus become important to explore the therapeutic potential variety of the plants of the area in a systematic manner.

It thus becomes important to survey and study systematically the availability of antimicrobial compounds in the plants which are used in traditional clinical practice or that are available in the local deserts area rich in ecophytodiversity.

The present work was under taken in the view of following **Objectives:**

1. **To evaluate the antimicrobial activity of selected plant extracts.**
2. **To measure the minimum inhibitory and minimum lethal concentration of the plant extracts.**
3. **To determine antifungal activity of some plant extracts.**

Review of literature

Review of literature

Antibiotics which are developed in the middle of 20th century and modified there after have been successfully used in limiting major bacterial illnesses. But the emergence of resistance against commonly used antibiotics has possessed challenge to clinical in treating mixed infections caused by predominantly antibiotics resistant organism. Continuous race between development of newer antibiotics and emergence and selection of resistance may result in to multiple antibiotics resistant **(Frost and O'Boyle, 1981)** super infection of bacteria which might be very difficult to kill. This has renewed the interest of researchers the world over to empirically determine the potential antimicrobial activity of many indigenous plants not explored. Plants and their products are used for the treatment of many illnesses in human and animals in all

parts of the world. Purified active principles of many indigenous plants are still practiced in modern medicine.

The Thar desert encompassing Bikaner and adjoining areas is rich in many unique flora which are important component of desert ecosystem Some of these plants are rich sources of nutrients and thus they are used by animals for grazing. They are found in the greater India which are selected, and also used by the physician from a long time for treatment of many disease. They have a lot of pharmacological activity from which selected only antimicrobial activity and antifungal activity. In the present study the potential antimicrobial activity of some of these plants was explored and the available literature on these plants is reviewed as under.

PLANTS DESCRIPTION:

1. **MORINGA OLEIFERA:**
Or MORINGA PTERYGOSPERMA
(Family: Moringaceae)

English: Horse radish tree, Drumstick tree
Hindi: Sahijan, Sahnjana, Sanjan, Mungana

It is an unarmed middle sized tree, with grayish brown trunk, and easily breakable branches; leaves usually tripinnate, rachises slender thickened and articulated at the base, leaflets elliptic or obviate, rounded at the apex, nerves obscure; 4-6 pairs and an odd one. Flowers are creamy white or yellow in colour. Capsule is acutely 3-quetrous and slightly constricted between the seeds. Seeds are 3-winged. This plant commonly grows in wasteland and cultivated in gardens and house. Fast growth can be achieved by cuttings **(Shetty and Singh, 1991).**

Duero (1984) studied the antibacterial activity of leaves of Moringa oleifera. Leaves extract was prepared by solvent extraction using ethyl alchohol and water as solvent

and antibacterial activity against both the organism in the from of zone of inhibition in the culture media.

Cacereers et al. (1991) found preliminary screening of antimicrobial activity of Moringa oleifera. Leaves, root and seeds were tested against bacteria, yeast dermatophytes and helminthes by using disc diffusion methods. The result showed that fresh leaf juice and aquous extracts of seed inhibit the growth of Pseudomonas aeruginosa and Staphylococcus aureus

When extraction temperature was above 56 ^{O}C, this activity was inhibited. No activity was observed against other pathogenic bacteria and Candida albicans. A method was standardized for studying the effect of aqueous extract on Ascaris-lumbricides eggs, but no activity was exhibited by any part of tree in contrast to Chenopodium ambrosioides leaf extracts.

Emeruwa (1991) observed antimicrobial activity of aqueous extracts from seed of Moringa oleifera against fungi including Candida and Penicillum and bacteria including Proteus, Streptococcus and Mycobacterium spp. Remarkable results were obtained against all the fungi and bacteria tested.

Singh et Al. (2003), observed antimicrobial activity of leaves, root, bark and seeds of Moringa oleifera against bacteria, yeast, dermatophytes and helminthes. The fresh leaf juice and water extracts tested against green algae, E. Coli, Pseudomonas aeruginosa, and Staphylococcus auerus, Bacillus sterothermophilus and Herpes simplex virus type I and Polio virus type I. The antibacterial effect of aqueous methonolic extract and water extract showed a fluctuation in its effects. Pseudomonas aeruginosa was more sensitive to all Moringa oleifera extracts; bacillus sterothermophilus was more sensitive than other organism to all extracts.

Dried leaves ground with garlic, salt, black pepper and turmeric are used as a treatment for dog bites or infections. Fresh leaf juice, mixed with honey, is used as an ointment for sore eyes. Decoction of dried leaves is taken orally for abortion and externally for rheumatism and wound healing.

Leaves are taken orally as an aphrodiasic and to treat wounds, the leaves are powered with turmeric and buttermilk and then applied **(R. N. Chopra, 1932)**. As it contains sulphur, it is recommended for rheumatism, ascites and venomous bites; as a poultice for neuralgia of the face. Ethanol extracts (95 %) of dried flowers, dried fruits, dried leaves and dried root, undiluted on agar plate, was active on Escherichia coli and Staphylococcus aureus.

Saline extract of leaves, at a concentration of 1: 20 on agar plate was active on Staphylococcus aureus and inactive on Pasteurella pestis. Fresh leaf juice at a concentration

of 100 micro liters on agar plate was active on Pseudomonas aeruginosa and inactive on Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes.

Water and hexane extracts of dried seeds, applied externally to mice at a dose of 10.0 %, were active on Staphylococcus aureus. Powdered dried seeds at a concentration of 100 micro liters, were active on Staphylococcus aureus and inactive on Escherichia coli, Pseudomonas aeruginosa and Streptococcus pyogenes.

Water extracts of dried seeds, at a concentration of 1: 10 on agar plate, was active on Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Sarcina lutea and Staphylococcus aureus. The extract was equivocal on Escherichia coli, Salmonella edinburgi and Serratia marcesens; inactive on Klebsiella aerogenes and produced weak activity on Proteus mirabilis and Streptococcus faecalis.

Powdered dried bark, powdered dried root, powdered dried seeds fresh leaves and powdered dried leaves at a concentration of 1 ml on agar plate, were inactive on Epidermophyton flaccosum, Microsporum canis, Microsporum gypseum, Tricophyton mentagrophytes and Tricophyton rubrum.

Water extracts of dried seeds at a concentration of 1: 10 on agar plate, was active on Botrytis allii, Coniophora cerebella, Penicillum expansum, Phytopthera cactorum, and Polyporus vesicular. The extract was equivocal on Fusarium oxysporum and inactive on Aspergillus oryza (Antifungal activity).

An extract of the entire plant, on agar plate was active on Mycobacterium tuberculosis. Water extract of dried seeds at a concentration of 1: 10 on agar plate, was active on Mycobacterium phlei **(Schramm, 1956).**

PHARMACOLOGICAL ACTIVITIES:

Antibacterial activity.

Anticonvulsant activity.

Antifertility effect.

Antifungal activity.

Antihemolytic activity.

Antihepatotoxic activity.

Antihistamine activity.

Anti-implantation effect.

Anti-inflammatory activity.

Antimalarial activity.

Antimycobacterial activity.

Antispasmodic activity (unspecified type).

Antitumor activity.

Antiyeast activity.

Barbiturate sleeping time decrease.

Carcinogenesis inhibition.

CNS depressant activity.

Diuretic activity.

Embryotoxic effect.

Hyperglycemic activity.

Hypoglycemic activity.

Hypocholesterolemic effect.

Hypoproteinemia activity.

Hypotensive activity.

Myocardial depressant activity.

Polygalacturonase inhibition.

Protopectinase inhibition.

Skeletal muscle relaxant activity.

Thyroid hormone effect.

Uterine stimulant effect.

Figure : I MORINGA OLEIFERA

2. WITHANIA SOMNIFERA :

DUNAL or

PHYSALIS FLEXUOSA

(Family: Solanaceae)

English : Winter cherry.

Hindi : Asgandh; Punir; Ashvagandha

The name Ashvagandha, means ‘‘the thing that has the smell of a horse ‘‘- a reference to the horse's strength and vitality rather than its odour. Ashvagandha is a wonder herb of India; regarded as 1st class adaptogenic tonic. It is a small woody herb belonging to family Solanaceae; an erect branching undershurb reaching about 150 cm in height, usually clothed with minutely stellate tomentum; leaves ovate up to 10 cm long; flowers greenish or lurid yellow in axillary fascicles; fruits globose berries which are orange coloured when mature, enclosed in a persistent calyx. The fleshy roots when dry are cylindrical, gradually tapering down with a brownish white surface and pure white inside when broken.

Constituents:

Withanolide (steroid).
Withasomnine.
Sitoindosides (glycowithanolides).
Withaferin A.
Sominiferin.
Withanine.
Anahygrine.
Pseudotropine.
Reducing sugar Phytosterol and Ipuranol.
Mixture of saturated and unsaturated acids.

The aphrodiac effects of Ashvagandha may take precedence over all its other outstanding properties. It should be considered as premiere herb for all negative condition associated with aging, **(Kuppuurajan, 1980)** this include its use in Alzheimer disease, arthritis **(Kulkarni R. 1991)** anti-inflammatory, antiseptic, antitussive, sedative and as a rejuvenative. It has a characteristic flavor due to presence of certain steroidal lactones. It is from this ordour which its Sanskrit name “like a horse “.

Ray and Majumdar (1976) studied the antimicrobial activity of different plant parts of 105 Indian species. Only 30 species showed antibacterial activity (of which 20 also had antifungal activity). These include roots of Withania somnifera.

Jaffer et al. (1988) studied the antimicrobial activity of Withania somnifera extract against different gram positive, gram negative and candida species and no antimicrobial activity against gram negative bacteria was observed. However leaf chloroformic, leaf methanolic and stem chloroformic extract displayed most significant antibacterial activity against gram positive bacteria.

Kazmi et al. (1991) analysed the antimicrobial activity of Withania somnifera. The crude extract of Withania somnifera inhibited the growth of Tricophyton mentagrophyte, Microsporium cannis and Aspergillus boydii at an MIC of 450 - 500 microgram / ml whereas pure compound inhibited the growth at MIC of 300 - 350 microgram / ml species of Corynebacterium, Bacillus, and Streptococcus spp. and Staphylococcus aureus were found to be highly susceptible to both crude and pure compound.

Ramadan et al. (1994) reported studies on alchoholic and aqueous extracts from 20 wild medicinal plants from the Qassim region. The sensitivity of 18 microbes (5 - gram positive and 6 - gram negative bacteria 5 - fungi 2 - yeasts) to the prepared extracts at concentrations of 10, 25, 50, 100 and 200 mg / ml was investigated. The MIC values for different active extracts were also investigated against the bacteria. Alcoholic and aqueous extracts of the studied parts exhibited strong antibacterial activity against Staphylococcus aureus, Staphylococcus aureus (methicillin resistant), and Streptococcus type B and D, Salmonella type C, Escherichia coli, Haemophilus influenzae, Proteus mirabilis and pseudomonas aeruginosa. The MIC values for Centaurea bruguierana, Rhazya stricta, Peganum harmala, Cynomorium coccineum and Withania somnifera extracts against H. influenzae were 8.36, 8.42, 8.47, 23.77 and 23.87 mg / ml respectively. Fungi and yeast were less sensitive, with the exception of Tricophyton mantagrophytes, which was slightly sensitive to some plant extracts.

Dhuley (1998) studied the therapeutic efficacy of Ashwagandha against exprimental aspergillosis in mice and found to have antifungal and immunomobulatory activites.

Bohra et al. (2002) studied the anti-microbial activity of different parts (root, stem, leaves, flowers and fruits) of Withania somnifera (alcohol and aqueous extracts) tested against Staphylococcus aureus, Streptococcus mutans, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli.

The stem, flower and fruits extracts showed highest inhibition of Staphylococcus aureus, Streptococcus mutans and Escherichia coli. The aqueous extracts of stem and flowers showed better inhibition of Staphylococcus aureus, Pseudomonas aeruginosa, while stem alcoholic extracts showed better inhibition of Escherichia coli. Flower and fruits extracts showed higher inhibition of Pseudomonas aeruginosa then other plant parts. Salmonella typhi was not inhibited by any of extracts.

Arora et al. (2004) studied the antibacterial activity of Withania somnifera by agar plate disc diffusion methods against Salmonella - typhinurium and E. coli using methanol and ether extract from both leaves and roots of Withania somnifera. The maximum inhibitory concentration assessed was 0.1 mg / ml for Salmonella typhinurium and E coli. From the extracts tested. Only methanol and hexane extracts of both leaves and roots were found to have potent antibacterial activity.

Mothana and lindequist (2005) selected 25 plants belonging to 19 families from different localities of the island Soqutra and extracted with solvents choloroform, methanol and hot water. The extracts were tested for their antimicrobial activity against one yeast species using agar diffusion method. Antimicrobial activity against several gram positive and several gram negative bacteria and one yeast species using agar diffusion method. Antimicrobial activity was demonstrated especially against gram positive bacteria including multiresistant Staphylococcus strains. The greatest activity was exhibited by the methanolic extracts of Withania adunensis and Withania riebeckii.

Owais et al. (2005) evaluated the antibacterial activity of Aswagandha (root and leaves). Both aqueous and alcoholic extraction of plants (root and leaves) were found to possess strong antibacterial activity against a range of bacteria by invitro agar well - diffusion methods. The methanolic extraction was further subfractionated using various solvents the butanolic sub fraction was found to possess maximum minimum inhibitory concentration against a spectrum of bacteria including Salmonella typhy-nurium. In constrat to synthetic antibiotics (Chloramphenicol), these extracts did not lead to lyses of human RBCs on incubation, advocating their safety to the living cell. Finally efficacy of the extracts isolated from plants (leaves and root) was determined against experimental Salmonella in bulb / c mice as revealed by increased survival rate**.**

PHARMACOLOGICAL ACTIVITIES:

An adaptogen.

Antianxiety effect.

Antiarthritic effect.

Antibacterial activity.

Anticonvulsant activity.

Antipyretic activity, analgesic, antiinflammatory activities.

Antispasmmodic effect.

Antistress agent.

Aphrodisiac activity.

Antisterility effects.

CNS depressant activity.

Haematics and growth promoters in growing children.

Hypotensive eefect.

Immunomodulatory activity.

Immunosuppressive effect.

In the management of amla -pitta.

Antiinflammatory and liver protective activities.

Tumouricidal activity and gastric cytoprotective effects.

Sedative and sleeping inducing effects.

Treatment of anaemia.

Figure II : WITHANIA SOMNIFERA

3. **CITRULLUS COLOCYNTHIS:**

(Family: Cucurbitaceae)

English : Colocynth, Bitter apple.

Hindi : Badi Indrayan, Mekkal , Visala, Mahendravaruni, Tumba.

Its Hindi name is tumba and is a perennial, and an extensively annual herbs with bifid tendrils, angular branching stems woolly tender shoots; leaves deeply divided and crisped, lobes narrow, thick, glabrous or some what hairy flowers monoecious, yellow, both male and females solitary, corolla pale yellow, fruit s globosely or oblong fleshy indehiscent berry and variegated with green and white; seed pale brown. It is found in desertic zone and traditionally used as a drastic purgative **(Shetty and Singh, 1991).**

Constituents:

Colocynthin.
A glucoside.
Colocynthein (Resin).
Colocynthitin.
Pectin.
Albuminoids.

Offonry and Achi (1998) investigated the microbiological characteristics in Melon seeds (Citrullus colocynthis). The pulp underwent a natural fermentation during 7 days exposure, was characterized by growth of Bacillus subtilis, Bacillus polymyxa, Lactobacillus fermentum, Streptococcus fecalis and significant contribution were made by Staphylococcus, Entrobacter cloacae, Penicillum, Aspergillus, Rhizopus species including the yeast. Growth of microorganism was completely inhibited in antibiotic treated samples indicating that the melon pods were the main source of microogranism for fermentation.

Adam et al. (2000) studied the effect of oral administration of tumba fruits alone and combined along with Rhazya stricta use in Najdi sheep. The result were indicating that the oral administration of 0.25 g/kg/day of tumba fruit or 0.25 g/kg/day of Rhazya stricta leaves for 42 days did not prove fatal but that mixture of both plants (0.25 g + 0.25 g/kg/day) proved fatal with profuse diarrhea, ataxia prior to death.

Memon et al. (2003) studied the antibacterial properties of Citrullus colocynthis against gram positive and gram negative bacilli using ethanolic extract of fruits, leaves, stem and their roots. Ethanolic extracts of fruits, leaves, stems and roots were found to be against gram positive bacilli, viz. Bacillus pumilus and Staphylococcus aureus while fruits and roots extracts in double strength gave positive results against bacillus subtilis. No activity was found against E coli. and Pseudomonas aeruginosa.

PHARMACOLOGICAL ACTIVITIES:

ROOTS :

Treatment of Uteralgia.

Treatment of Mammillitis.

Treatment of Rheumatalgia.

Treatment of Visceromegaly.

Treatment of Ophthalmia.

Treatment of Ascites.

Treatment of Jaundice.

Treatment of Uropathy.

FRUITS :

Purgative.

Antipyretic.

Anthelmintic.

Treatment of Tumours.

Treatment of Ascites.

Treatment of Leucoderma.

Treatment of Ulcers.

Treatment of Asthma.

Treatment of Bronchitis.

Treatment of Urethrorrhea.

Treatment of Jaundice.

Treatment of Dyspepsia.

Treatment of Constipation.

Treatment of Elephantiasis.

Treatment of Tubercular glands of the neck.

Treatment of Splenomegaly.

Treatment of Migraine.

Treatment of Scorpion sting and Snake bite.

Figure III : CITRULLUS COLOSYNTHIS

4. SALVADORA OLEOIDS :

(Family : Salvadoraceae)

English : Tooth Brush Tree.

Hindi : Pilu, Kankhina, Jhal, Kharkanella, Khara jhal.

A shrub or occasionally a small tree with a short twisted or bent trunk; branches numerous, stiff, divergent, whitish. Leaves are whitish green, coriaceous and some

somewhat fleshy when mature, linear lanceolate, acute or sub obtuse, often mucronate, glabrous; main nerves indistinct. Flowers are greenish white, sessile, in errect axillary panicled spikes, often clustered. The fruits have a sharp, pungent, acrid and sweet, sour taste with a flavour.

Constituents:

Alkaloids Trimethylamine.

Aromatic oil and fixed oil.

Akpata and Akinrimisi (1977) found the antibacterial activity of some extract from some African Chewing stick including Salvadora Species which inhibited the growth of periodontal pathogen Porphyromons gingivalis and Bacteriodes melaninogenus invitro.

Albaghieh et al (1994) observed the antimycotic effect of the aqueous extracts of roots of salvadora oleoids several concentration of aqueous extracts of miswak prepared with Sabourauds medium were inoculated and incubated at 37 ^{O}C and turbidity was determined at 600 nm wavelength measured at specific interval over a period of 48 hours. At a concentration of 15 % the extract had fungi static effect for up to 48 hours.

Allafi and Ababneh (1995) described the effect of extract of miswak (Chewing sticks) used in Jordan and middle east Arabia on oral bacteria. Three methods of determining antibacterial activity were carried out as streaked plate method, disc plate method, tube dilutation method for MIC. It was found that extract of these sticks had a drastic effect on growth of Staphylococcus aureus with MIC of 69 mg/ml/100CC.

Al-samh and Al-bagieh (1996) observed the antibacterial and antifungal effects of an ethanolic extract of Salvadora oleoids (miswak) and compared it with the similar activity of sodium hypochlorites (NaClO). Various concentration of NaClO was taken and extract were prepared and inoculeted with the Streptococcus faecalis and Candida albicans, and incubated at 37 ^{O}C for 7 days. The turbidity was determined daily using a spectrophotometer. 5 % and 1 % NaClO and the extract (200 mg/ml) had bactericidal and antimycotic effects. Whereas a 50 % concentration of extract (100 mg/ml) had a bactericidal and fungi static effect up to 48 hours, 25 % (50 mg/ml) extract showed no bactericidal or antifungal properties.

Ahmad (2001) studied the antimicrobial effect of Salvadora oleoids from India. He used the disc plate method, to test the antimicrobial and fungicidal activity of different plant extracts. The inhibition zone up to 1.8 mm was found in the extract of Salvadora plant.

Alali et al (2004) determined the antimicrobial activity of volatile oil and aqueous and alcoholic extract of the Salvadora oleoids. Among all test fractions the volatile oil exhibited potent activity against Pseudomonas aeruginosa and Staphylococcus aureus.

Krishanan (1998) studied the antimicrobial activity of Salvadora oleoids. He used above methods along with it buffering capacity and fluoride contents from the aqueous and alcoholic extacts of plant parts.

PHARMACOLOGICAL ACTIVITIES:

Treatment of painful Rheumatic affections (Stimulating effect).

Stomachic.

Vesicant.

Purgative. (Given to horses).

Aphrodisiac activity.

Treatment of Enlarged Spleen.

Treatment of Rheumatism and low fever.

Treatment of Snake bite.

Appetizer.

Carminative.

Alexipharmic.

Treatment of Piles.

Treatment Tumors.

Treatment of Bronchitis.

Treatment of Ascites.

Figure IV : SALVADORA OLEOIDS

5. SWERTIA CHIRATA

(Family - Gnetianaceae)

English : Swerita chirata

Hindi : Chirayata, Mamajaka, Meetha kirayata,

Swerita chirata is an erect annual herb found throughout the greater part of India. The stem, which attains about 6 mm in thickness, is of a yellowish brown colour, glabourous, slightly winged. The lower part of the stem is rounded, and the upper part of the stem produces in the axils of the opposite leaves numerous slender, elongated bearing fruits and occasional flowers. The drug has no marked odor, but all parts have an extremely bitter taste.

Constituents:

Ophelic acid

Chiratin

It is used in indigenous medicines in the treatment of fevers and as bitter tonic and forms one of ingredients of many hypoglycemic-marketed formulations. According to Ayurvedic literature survey, the fresh juice of leaves has been used as a bitter tonic, to control arthritis, in typhoid fever and as a cooling agent. It is as stomachic and laxative, blood purifier in dropsy, rheumatism, abdominal ulcers, hernia swellings, itches, and insect poisoning. The plant paste is applied on boils.

The leaves are fed to cattle to increase appetite. Plant extracts were reported for the biological activities such as antidiabetic, anti-inflammatory, stimulant, astringent and diuretic and anthelmintic propertite.

It also acts as ethno medicine for snake bite. The plant is used to cure leucorrhoea. The root extracts showed antimalerial activity both invitro and in vivo. Methanolic extract showed antidiabtic effect in alloxon induced diabetic rats. It inhibited carrageen-induced edema and its anti-inflammatory activity is comparable to that of hydrocatisone. The plants are extremely bitter due to bitter principle Ophelic acid and amrogentian.

PHARMACOLOGICAL ACTIVITIES:

Antispasmodic effect.

Anti-inflammatory activity.

Antipyretic activity.

Immunostimulant activity.

Antihepatotoxic activity.

Antidiabtic activity.

Anti carcinogenic activity.

Antimalarial activity.

Bitter tonic.

Figure V : SWERITA CHIRATA

MATERIALS AND METHODS

MATERIALS AND METHODS:

1. MATERIALS

A). Ingredients of bacteriological media:

I. Agar -agar type I (Hi - media lab Pvt. Ltd.)

II. Beef extract (Glaxo - Lab Chemical Division)

III. D - Mannitol (Hi - media Laboratories)

IV. Dextrose sugar (Hi - media Lab Pvt. Ltd)

V. D - lactose (Sarabhai M. Chemicals)

VI. Eosin Water Soluble Yellowish (George T. Gurr Ltd)

VII. Mc - conkey Agar Base (Hi - Media Lab. Pvt. Ltd.

VIII. Methylene Blue M. S. (S. D. Fine Chem. Pvt Ltd.)

IX. Peptone- Bacteriological (Glaxo Lab Chemical Divison)

X Sodium Chloride (Glaxo Lab Chemical Divison)

B). Chemicals and Reagents:

I. Alpha napthol

II. Barium Chloride Powder

III. Buffer tablets pH 7.0 (Glaxo Lab and Fine Chemical)

IV. Crystal violet (Glaxo Lab and Fine Chemical)

V. Ethyl alcohol.

VI. Heparin sodium injection, 25000 I U in 5 ml (Biological Ltd).

VII. Kovac's Reagentes.

VIII. Methyl Red Indicator.

IX. Neutral red.

X. Phenol- red -pH indicator.

XI. Potassium hydroxide.

XII. Potassium iodide.

XIII. Sulphuric acid.

XIV. Xylene (Glaxo laboratories, chemical division)

C). Other materials:

I. Distilled water

II. Hi - Media antibiotics disc.

III. Mastitis cattle milk samples and calf diarrhea sample.

IV. Normal saline solution.

V. Rabbit plasma.

VI. Selected indigenous plant parts.

VII. Sheep blood.

2. Preparations of stains:

A large number of coloured compounds (dye) are available for staining microorganisms. These compounds are generally rather complex in terms of molecular structure. Fixed staining preparation are most frequently used for the observation of the morphological characteristics of bacteria. The advantages of this procedure are that;

1). the cells are made more clearly visible after they are coloured.

2). Differences between cells of different species and with in the same species can be determined by use of appropriate staining solutions (differential or selective staining).

I. Stain for Gram's staning:

Gram staining is one of the most important and widely used differential techniques. This technique was introduced by Christian Gram in 1884. In this process the fixed bacterial smear is subject to the following staining reagents in the order listed.

(a). Crystal violet (Primary stain)

1 % crystal violet aqueous solution.W / V

(b). Neutral red

1 gm of neutral red dissolved in 2ml of 1 % acetic acid solution made to 1000 ml in distilled water.

(c). Gram's iodine solution

20 gm of potassium iodide and 10 gm of iodine crystals dissolved in 1 L of distilled water.

II. Lacto phenol cotton blue stain:

Phenol crystal 20.0 g, Glycerin 20.0 g, Lactic acid 20.0 g and Water 20.0 ml were mixed with gentle heating. Cotton blue 0.05 g added and dissolved.

3. Preparation of bacteriological media:

I. Nutrient agar

10 g of peptone was mixed with 5 g of beef extracts and 5 g of sodium chloride. These ingredients were mixed to 1000 ml of distilled water and pH was adjusted to 7.2. Agar - agar type I was added at the rate of 2 %. The media was autoclaved at 121 ^{O}C and 15 lb pressure, dispersed in Petri dish and stored in refrigerator at 4 ^{O}C till use.

II. Blood agar

Nutrient agar basal media was prepared and autoclaved. The temperature of the medium was brought to 50 ^{O}C and sheep blood was added at rate of 5 % and then dispersed in Petri dishes.

III. MacConkey agar base media

Readymade MacConkey agar base media (Hi Media) was used. Lactose was sterilized at 10 lb pressure in autoclave and added to basal media.

IV. Mannitol salt agar

Beef extract 1 g; Peptone 10g, Sodium chloride 75 g and Phenol red 0.025 g were mixed and dissolved in 1000 ml of distilled water, then pH was adjusted to 7.4. Then

10 g of mannitol was sterilized in water bath at 90 ^{o}C for 30 minutes and added to basal media.

V. Hugh and Leifson's medium

2 g of Peptone, 5 g of Sodium chloride, 0.3 g of di - basic Potassium phosphate, and 1 % Bromothymol blue (3ml) were mixed in 1000 ml of distilled water. Agar - agar at rate of 0.5 % was added and then pH was adjusted to 7.1. Glucose was added to the final concentration of 10 %. This medium was autoclaved and distributed in tubes.

VI. Eosin methylene blue agar

Peptone 10 g, lactose 10 g, Di potassium hydrogen phosphate 2 g, Eosin yellow 0.4 g, and Methylene blue 0.065 g were dissolved in 1000 ml of distilled water. Agar - agar was added at rate of 2 % and final pH was adjusted to 6.8. The media was autoclaved at 121 ^{o}C for 15 minutes at 15 lb pressure.

VII. Sabouraud's dextrose agar

Dextrose sugar 10 g, Peptone 10 g, and Agar 20 g mixed in 1000 ml distilled water, and then pH was adjusted to 5.0 - 6.0 and the media was autoclaved at 121 ^{o}C for 10 minutes at 10 lb pressure.

4. METHODS

The proceeding of the methodology was as follow:

A. Collection of plants

Antimicrobial activity of plants was carried out by testing them against gram positive bacteria and fungi. The following plants were selected for determination of antimicrobial activity.

1. Moringa oleifera

2. Withania somnifera

3. Citrullus colocynthis

4. Salvadora oleiodes

5. Swerita chirata

All the above mentioned plants were collected from College of Veterinary and Animal Science campus, Bikaner, Rajasthan Agricultural University campus, Beechwal, Bikaner and herbal medicinal plant library, Dungar college, Bikaner and herbal medicinal garden, Jhalrapatan and identified by dept. of botany, Dungar college, Bikaner. The following parts from the plant were collected for determimnation of antimicrobial activity.

1. Moringa oleifera : Leaves

2. Withania somnifera : Leaves

3. Citrullus colocynthis : Fruits

4. Salvadora oleiodes : Leaves

5. Swerita chirata : Leaves

Collected plant parts were washed and cleaned by muslin cloth and kept for drying for 7 days at 40 ^{O}C. Then plant parts were ground in to a powder form.

B). Extraction of plant phytochemical

Extraction may be defined as the process of removal of removal of desirable soluble constituent from a substance, leaving out those which are not wanted, with the aid of solvent and standardized processes.

Extraction is a process in which generally a part is treating with solvent for separating out the active constituents completely or partially.

Plant contains chemical substances some of which provide relief and a variety of diseased conditions. The isolation of active constituent may be an extremely difficult and expensive process. If the other constituents have no undesirable effect, the administration of the unprocessed drug or its partially purified extracts may provide the

desired therapeutic effect. In recents years active principles from both plants have been isolated or obtained as purified products of precisely known potency and stability.

The solvent used for extraction is known as "Menstruum" and the undissolved residue left behind after the process is called "Marc". The process of drug extraction can be summarized in to these steps.

1. **Penetration of the solvent in to the drug.**
2. **Dissolution of the constituent.**
3. **Outward diffusion of the solutions from the cells.**
4. **Separation of dissolved portion.**

I. Preparation of Aqueous extract

Aqueous extraction was carried out by decoction process. This was carried out by boiling in hot water. In this process I part of dried powder of plant and 5 part of sterilized distilled water were taken in a boiling water flask and boiled for 15 minutes. After boiling the extract was filtered through a What man filter paper no. I, autoclaved at 121 ^{o}C for 15 minutes and kept in clean and sterilized test tube and stored at 4 ^{o}C till further use.

II. Preparation of alcoholic extract

Alcoholic extract of indigenous plants were prepared according to the methods described by Davis. The Alcoholic extract was prepared by continuous hot percolation process which is known as "Soxhlet Extraction". In this process the dried powder form of plant material was extracted by using a little volume of a hot menstrum repeatedly. The hot menstrum was ethyl alcohol and it extracted out the active components of plant when it repeatedly passed through a packed column of plant material. The apparatus used for continuous hot percolation process is known as Soxhlet apparatus and process is known as Soxhlation.

The dried and grinded plant material to be extracted was packed in a cylinder made from a filter paper and placed in an extractor. Precaution was taken that the upper position of the plant material remained below the upper part of the siphon. The solvent or menstrum i.e. Ethyl alcohol was in boiling flask. On heating the menstrum was converted in to vapour. The vapour entered in to condenser through the

side tube and condensed there. The condensed hot Ethyl alcohol fell on the packed column of the plant material and extracted out the ingredients of plants which moved downward through the packed column and collected in an extractor.

As more and more menstrum passed through the packed column of plant material, the level of liquid in extractor as well as in the siphon went on increasing. When the level in the siphon reached at the highest position, it carried down the extract from extractor to flask. On further heating, the vapour of Ethyl alcohol left the flask while the soluble active constituents remained in it. The process of filling and emptying of the extractor was repeated for 14 - 15 times and it required 4 - 6 hours, for the complete extraction of active constituent from the plant material. After completion of the process the concentrated active constituents from plant material were kept in sterilized test tubes stored in refrigerator till further use. The traces of ethanol were removed by keeping the tubes at 50 ^{o}C for 1 hour.

5. Isolation and identification of test bacteria

Milk samples from 4 mastitic cattle and one fecal sample from calf diarrhea were screened for the presence of bacteria by cultivation, isolation and identification using standard procedure of Cown and Steel (1975). The mastitis cattle milk sample and calf diarrheal sample were withdrawn with an inoculating loop aseptically and streaked on blood agar, nutrient agar and MacConkey agar culture media plates in primary, secondary and tertiary fashion in order to obtain isolated colonies of bacteria. These Petri plates were inoculated for 24 hours at 37 ^{o}C and if colonies did not appear or were found to be small, the plates were incubated for further 24 hours.

Following incubation, the plates were observed for colonies characteristics and haemolytic zone on blood agar plates; the different colonies were selected out and subculture separately for obtaining the pure culture of the bacterial isolates.

The smear were prepared from the bacterial pure colonies, fixed by gentle heating and stained by Gram's methods. The stained smear was examined under oil immersion objective for determining Gram's reaction, morphological characteristic so as to ascertain homogenicity of the organism. The pure isolates were taken on nutrient agar slants and preserved in a refrigerator at 4 ^{o}C until subjected to further biochemical characterization.

MacConkey agar culture media plates were observed for the appearence of pink coloured colonies. Idenificaion of the pathogen was done by carrying out of the following procedure :

Primary the smear were prepared from the bacterial pure colonies, fixed by gentle heating and stained by Gram's methods. The stained smear was examined under oil immersion objective for determining Gram's reaction, morphological characteristic so as to ascertain homogenicity of the organism.

Primary identification up to generic level

I. Morphology

Colonies of bacteria on nutrient agar plate were purified and bacteria were observed for thier size, shape arrangement, sporulation, capsulation and presence of any other distinctive feature.

II. Motility

Motility was studied in hanging drop preparation of broth culture of bacteria.

III. Growth in air

Growth in air was studied to confirm whether the bacterial isolates were able to grow under aerobic or anaerobic condition.

IV. Acid fastness

Acid-fast staining property was determined by Ziehl Neelson method as per the technique of **John (1977).** Acid - fast reaction of bacterial cells was recorded as ' + ' and non acid fastness was recorded ' - '.

V. Gram's Reaction

Smear of young culture of bacterial isolates were stained by modified Gram's Method of staining described by **Hucker and Cohn (1923).** The results were noted as Gram positive (+) for organisms staining blue and Gram negative (-) for those isolates taking pink colour of counter stain.

VI. Spore - formation

Spore - formation was observed in smear prepared from colonies.

VII. Catalase Activity

Catalase activity was tested for the confirmation of bacteria producing catalase and the technique of **Thomas (1963)** was adopted. One ml of three percent solution of hydrogen peroxide was placed on a clean glass slide. Pure culture was picked up from nutrient agar slant with an inoculating straight wire in front of flame and placed on drop of reagent on glass slide. Culture was properly emulsified and cover slip was placed. The production of gas bubble confirmed a positive reaction.

VIII. Oxidase Activity

It confirms the production of cytochrom oxidase by certain bacteria. The culture from nutrient agar slant was picked up with an inoculating loop and rubbed on filter paper. Simultaneously a drop of oxidase reagent (N, N, N, N - p, Phenylene - diamine dihydrochloride) was added. Colonies producing oxidase gave coloured reaction, the colour of filter paper turning to deep blue in a few seconds.

IX. Oxidation and fermentation test

This test was used to differentiate oxidative bacteria from fermenters following the technique of Hugh and Leifsons. Hugh and Leifsons medium was used containing glucose and bromothymol blue as indicator. Semisolid medium was inoculated in pairs by culture of bacteria to be tested. One tube of pairs was kept open, while the others tube was covered with 1 - 2 mm l ayer of sterilized paraffine to provide anaerobic condition.

The tubes were inoculated for 24 hours. Those bacteria that oxidised the sugar showed acid production and yellow discolouration of the medium in open tube. Bacteria that ferment the sugar showed acid production and yellow discoloration in both the paired tubes.

Secondary identification test and use of selective media (up to species level)

The above set of test was applied for each of the bacterial isolates and the results were recorded as positive (+) and negative (-). The generic status of each bacterial isolated was attemped by comparing the set of tests with the standard

generic identification reactions recommended by Cown and Steel (1975) for gram's positive and gram's negative bacterial genera.

I. Coagulase test

Haemolytic bacterial colonies from blood agar were isolated and preserved on nutrient agar slant. These were tested for the confirmation of Staphylococcus. Coagulase production by Staphylococcus is an important criterion of its pathogenecity which was evaluated by the coagulase test.

(a) Collection of plasma

Rabbit blood was taken aseptically in tube containing 0.5 ml of heparin sodium by intra cardiac puncture. The tube containing blood was centrifuged at 2500 rpm for 15 minutes to separate the plasma. Clear plasma supernatant was taken in to another sterilized test tube.

(b) Tube coagulase test

Plasma was diluted 1: 5 in saline solution and 0.5 ml of plasma was taken in a sugar tube and culture of organism from slant was fished out and inoculated in to plasma and mixed thoroughly. The tubes were inoculated in water bath at 37 ^{O}C and were observed at 1, 4, and 8 hours intervals. A partial clotting of plasma was considered positive test.

II. Identification of selective media

(a) Mannitol salt agar

Mannitol salt agar plates were streaked with the test culture from slants and incubated for 24 to 28 hours. The mannitol fermenting pathogenic organism caused yellow discoloration of the media due to production of acid by mannitol fermentation which decolorized phenol red indicator to yellow, whereas the colonies of non pathogenic cocci were small and red.

(b) Eosin Methylene Blue agar (EMB)

Enteric bacteria were identified by Eosin methylene blue agar media. Pure lactose fermentation culture were streaked on EMB plates and incubated for 24 hours. Appearance of metallic colour sheen was considered as E. Coli.

6. Biochemical Test

I. Indole Test

Indole test was conducted to access the ability of bacteria to decompose amino acid trytophan to indole. Peptone water was inoculated with isolated test culture and incubated for 48 hours at 37 ^{O}C. After incubation, 0.5 ml of Kovac's reagent was added and shaken gentle. Appearance of red colour indicated positive reaction.

II. Methyl Red (MR), Voges Proskaure (VP) Test

MR - VP broth was prepared and inoculated with test culture and incubated for 4 days. To 5 drops of methyl red indicator were added. A positive reaction was shown by red colouration, indication of production of acid bringing pH down to 4.0.

III. Voges Proskaur (VP) Test

VP test was performed to detect the formation of acetyl - methyl - carbinol which is an intermediate product of carbohydrate metabolism. This test was performed after the conduction of methyl red (MR) test. First, 5 ml of 10 % KOH solution was added to neutralize the acidity, produced in MR test. Later 1 ml of 5 % L - naphthol reagent was added to broth culture. The tubes were shaken well and kept undisturbed for 5 - 10 minutes.

Brick red colour appeared in tube and dissolved through the broth, if acetyl - methyl - carbinol was produced.

IV. Citrate Utilization test

This test was carried out to test the ability of an organism to utilize citrate at the sole source of carbon. Simmon's citrate agar was used. The pure colony cultures from nutrient agar slant were fished out and streaked over citrate agar plates and incubated for 24 hours.

Appearance of blue colour was considered positive while original green colour of media showed that citrate was not utilized.

V. Nitrate Reduction

It is a test to determine the presence of the enzymes nitrate reductase which cause reduction of nitrate and tested by appropriate colorimetric reagent. Nitrate broth was inoculated with the test culture and incubated for 2 - 3 days. To 5 ml of broth culture was added 0.1 ml of test reagent. A red colour developing within a few seconds indicated the presence of nitrate and hence the ability of organism to reduce nitrate.

VI. Antibiogram determination

The following antibiotics discs (Hi-Media) were used for determination of the isolates.

Ampicillin	(A)	10 mcg
Amoxycillin	(Am)	10 mcg
Chloramphenicol	(C)	30 mcg
Doxycycline	(D)	30 mcg
Kanamycin	(K)	30 mcg
Bacitracin	(B)	10 unit
Gentamycin	(G)	10 mcg
Penicillin	(P)	10 unit
Vancomycin	(Va)	30 mcg
Sulfadiazine	(Sz)	300 mcg
Neomycin	(N)	30 mcg
Ciprofloxacin	(Cf)	5 mcg

Antibiogram of the culture isolates were based on the Bauer et all (1966) disc method. Nutrients broth in tubes was inoculated with the bacterial culture

from slants. After 6 - 7 hours, when the bacteria were in exponential phase of growth, the broth culture was swabbed on the Muller - Hinton agar plates by sterile cotton swab. When broth culture was dried, eight antibiotics discs were placed with the aid of automatic disc dispensor in front of flame.

The petriplates were incubated for 15 - 20 hours and observed for the zone of inhibition. The diameter of zone of inhibition was determined with the help of measuring scale and compared with the standard scale of inhibition for each antibiotics disc as per the instruction provided by manufacture (Hi-Media).

7. Determination of concentration of test organisms

The concentration (total count) of test bacteria (S.aureus and E.coli.) Was determined by nephelometry using McFarland scale (McFarland, 1977). The standard tubes were prepared by mixing varying amount of 1 % barium chloride and 1 % sulphuric acid in last stopper tubes as follows.

Table: McFarland scale

Scale	1% $Bacl_2$ (ml)	1% H_2SO_4 (ml)	No. of bacteria value listed x 10^6 approximately
1.	0.1	9.9	300
2.	0.2	9.8	600
3.	0.3	9.7	900
4.	0.4	9.6	1200
5.	0.5	9.5	1500
6.	0.6	9.4	1800
7.	0.7	9.3	2100
8.	0.8	9.2	2400
9.	0.9	9.1	2700
10.	1.0	9.0	3000

The turbidity of over night broth culture of test organism was compared with that of McFarland scale tubes against white back ground and concentration was approximated according to the table.

8. Preparation of dilution of plant extracts

Two fold serial dilution of aqueous and alcoholic plant extracts were prepared in sterilized test tubes with sterile normal saline solution beginning from 1:1 undiluted and 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512. The total amount of each dilution was kept 2 ml.

I. Inoculation of test organisms

Each dilution of plant extracts was added with equal volume of double strength (2x) of nutrient broth so as to make normal concentration of nutrients after the addition of the medium. Along with the desired plant extracts two set of control tubes were simultaneously taken one of which lacked plant extracts the other one was kept for un-inoculated control.

All the dilutions of plants extracts and control tubes (except UN - inoculated control tubes) were inoculated with 0.1 ml of broth culture of test organism having turbidity comparative to McFarland tube no. 1 with approximated number of organism as 300×10^6 per ml. Following inoculation all the tubes along with the control tubes were incubated at 37 ^{o}C. Turbidity of all the tubes was measured after 2, 4, 6, 8 hours. The highest dilution of plant extracts that showed inhibited growth of test organism as compared with the control was considered as MIC and determined using the methods adopted by **Tsuchiya et al (1996)**.

II. Determination of MLC

After 8 hour of incubation a series of 10 folds serial dilution of each inoculated tubes was prepared in sterile normal solution and were spread evenly on nutrient agar Petri plates with the help of sterile spreader. The plates were incubated at 37 ^{o}C over night and following the number of colonies were counted on colony counter to utilized live number of bacteria present. The highest dilution of the plant

extracts that was found to kill the test organism was considered as per the methods of **Sato et al (1997**).

9. Isolation and identification of test fungi

Sheep nasal swab was taken and cultured on Sabouraud's dextrose agar and the plates were incubated at room temperature for 2 days. Grayish brown mycelia were seen which turned later to black colour. Smear was prepared and stained with lacto phenol cotton blue stain and observed under high power microscope.

I. Preparation of spores

10 ml of sterile normal saline solution was added to the Petri plates containing fungal growth i.e. Showing fungal mycelium and was shaken gently. The fluid containing fungal spores was collected. With the help of sterile pipette, and centrifuged at 1500 rpm for 10 minutes. The supernatant was discarded and the sediment was washed twice with normal saline solution. Finally the spore sediment was suspended in normal saline at a concentration of about 100 - 200 spores per high power field.

II. Germination of spores

To a glass cavity slide (normally used for hanging drop preparation), 30 ml of Sabouraud's dextrose agar (0.5 % agar) was placed in a cavity portion while in molten (at temperature 50 ^{O}C) state. It was then added with 10 ml of test fungi spore preparation under possibly aseptically conditions. The cavity portion of the slide was covered with a sterile and clean cover slip and its margins were sealed with sterile paraffin wax. It was then incubated for two days in moist chamber at room temperature and observed under low power microscope for germination of spores.

III. Inhibition of spore germination activity

For determination of antifungal activity of plant extract, two folds serial dilutions (1:1 to 1:512) of aqueous and alcoholic extracts of test plants were prepared

and 40 micrometer of each dilution were added along with inoculation of spores as described above in spore germination techniques. The micro culture slides were incubated at room temperature in moist condition and observed for inhibition of spore germination at 24 hours and 48 hours of inoculation following the method described by Rana et al, (1997).

RESULTS

RESULTS

The present investigation involves aqueous and alcoholic extraction of some of the plant commonly found in this area. The aqueous and alcoholic extracts were used to determine their antibacterial and antifungal potential using pathogenic Staphylococcus aureus and Escherichia coli as test bacterial and Aspergillus spp. as test fungi. The results obtained are presented as follows:

I. Isolation and identification of test bacteria

(A). Isolation and identification of Staphylococcus aureus

Staphylococcus aureus could be isolated and identified from mastitic milk sample of cattle. The biochemical and metabolic property of Staphylococcus aureus isolated are presented in Table.

Table 1: Biochemical and metabolic reaction of Staphylococcus aureus

S.No.	Primary Identification		Secondary	

			identification	
1.	Gram reaction	+	Growth in MSA	+
2.	Morphology	Cocci	Coagulase	+
3.	Motility	-	Growth in EMB	-
4.	Spore	-	Indole test	-
5.	Growth on McConkey agar	-	MR test	+
6.	Catalase	+	VP test	+
7.	Oxidase	-	Citrate utilization	-
8.	O-F test	F	Nitrate reduction test	+

(B). Isolation and identification of Escherchia coli

Escherichia coli could be isolated from faecal sample of calf diarrhoea. The biochemical and metabolic property of Escherichia coli isolate are presented in Table

Table 2: Biochemical and metabolic reaction of Escherichia coli

S.No.	Primary Identification		Secondary identification	
1.	Gram reaction	+	Growth in MSA	+
2.	Morphology	Rods	Coagulase	+
3.	Motility	-	Growth in EMB	Metallic sheen
4.	Spore	-	Indole test	-
5.	Growth on McConkey agar	-	MR test	+
6.	Catalase	+	VP test	+

7.	Oxidase	-	Citrate utilization	-
8.	O-F test	F	Nitrate reduction test	+

II. Isolation and identification of test Fungi

The test fungi i.e Aspergillus fumigatus could be isolated from nasal swab of sheep. The identification of Aspergillus was based on its cultural and morphological characteristics. Their appearance were white puffy colony when it first appeared, rapidly become velvety, granular, green blackish in colour and hyphae showed parallel wall and dichotomous branching often showing ballooning.

III. Antibiotic sensitivity pattern of test bacteria

The test organism that is Staphylococcus aureus and Escherichia coli isolates were subjected to their sensitivity and resistance pattern to commonly used antibiotics. The results of antibiotic sensitivity pattern of test bacterial isolates are presented in Table.

Table 3 Antibiotic sensitivity and resistance of different test organism in sensidisc diffusion test

S. No.	Test Organism	Resistant	Sensitive
1.	Staphylococcus aureus	Amoxycillin, Ampicillin, Ciprofloxacin, Doxycycline, Methicillin, Penicillin, Sulfadizine	Bacitracin, Chloramphenicol, Ciprofloxacin, Gentamicin, Kannamycin, Neomycin, Vancomycin
2.	Escherichia coli	Amoxycillin,	Amoxycillin,

		Bacitratin, Ciprofloxacin, Penicillin, Sulfadizine, Vanocomycin, Ciprofloxacin	Chloramphenicol, Ciprofloxacin, Gentamicin, Kannamycin, Methicillin, Neomycin

IV. Extraction of plant phytochemicals

Extract could be prepared from five selected plant using hot water and Ethanol as a solvent. From 15 g of each plant part 4 - 5 ml aqueous extract could be prepared. In alcoholic extracts after removal of traces of ethanol 2-3 ml extract was left from each plant which was dark coloured and pasty in consistency and thus it was found difficult to measure the turbidity of dilutions of the extract before and after inoculation.

V. Antibacterial and antifungal activity of plant extracts

The alcoholic and aqueous extracts of selected plant were tested for antibacterial and antifungal activity. The results are presented in table as follows:

1. Moringa oleifera : Table No. IV
2. Withania somnifera : Table No. V
3. Citrullus colocynthis : Table No. VI
4. Salvadora oleoides : Table No. VII
5. Swertia chirata : Table No. VIII

The antibacterial activity of these plant extracts has been summarized in Table No. IX and antifungal activity in Table No. X.

VI. Antibacterial activity of aqueous extracts of selected plant

The aqueous extracts of selected plants were tested for antibacterial activity by turbidity method (MIC). The results are presented in Table No. XI. The comparative results of MIC and MLC of aqueous plant extracted has been summarized in Table No. XII.

Table No. IV

Antibacterial and Antifungal activity of Moringa Oleifera

Leaf Extracts

Type of extract	Organism	Plate count in control tube (ml)	Concentration of the Moringa oleifera leaf extracts									
			Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Alcoholic Extracts	E.Coli	600 x 10^6	0.78 x 10^6	1.3 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Staphylococcus aureus	600x10^6	0	0	0	0	0	0.06 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6

	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Aqueous Extracts	E.Coli	600 x 10^6	0	1.09 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Staphylococcus aureus	600 x 10^6	0.2 x 10^6	0.30 x 10^6	0.39x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table No. V

Antibacterial and Antifungal activity of Withania somnifera Leaf Extracts

	E.Coli	600 x		1.23 x	600 x	600 x	600 x	600 x	600 x	600 x	600 x	600 x
Type of	Organism	Plate	0	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6	10^6
Aqueous Extracts	Staphylococcus aureus	10^6 count in control tube (ml)	0.22 x 10^6	0.79 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Aspergillus spore		Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Alcoholic Extracts	E.Coli germination	600 x 10^6 Absent	0.66 x 10^6 Absent	Absent	600 x 10^6 Absent	600 x 10^6 Absent	600 x 10^6 Absent	600 x 10^6 Absent	600 x 10^6 Absent	600 x 10^6 Absent	600 x 10^6 Absent	600 x 10^6 Absent
	Staphylococcus aureus activity	600x10^6	0	0	0	0	0	0	0.52 x 10^6	6.96 x 10^6	600 x 10^6	600 x 10^6
	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Concentration of the Withania somnifera leaf extracts

Table No. VI

Antibacterial and Antifungal activity of Citrullus colocynthis furits extracts

Type of extract	Organism	Plate count in control tube (ml)	Concentration of the Citrullus colocynthis furits extracts									
			Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Alcoh	E.Coli	600 x 10^6	0.96 x 10^6	1.28 x 10^6	1.9 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6

olic Extracts	Staphylococcus aureus	600x10^{6}	0.1 x 10^{6}	0.12 x 10^{6}	0.23x 10^{6}	0.46x 10^{6}	600 x 10^{6}	0.06 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}
	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Aqueous Extracts	E.Coli	600 x 10^{6}	0.6 x 10^{6}	0.78 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}
	Staphylococcus aureus	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}	600 x 10^{6}
	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table No. VII

Antibacterial and Antifungal activity of Salvadora oleoides leaf extracts

Type of extract	Organism	Plate count in control tube (ml)	Concentration of the Salvadora oleoides leaf extracts									
			Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Alcoholic Extracts	E.Coli	600 x 10^6	0	1.28 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Staphylococcus aureus	600x10^6	0	0	0	0	0	0.77 x 10^6	0.90 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6

	Aspergillus spore germination activity	Absent	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Aqueous Extracts	E.Coli	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Staphylococcus aureus	600 x 10^6	0	0.8 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table No. VIII

Antibacterial and Antifungal activity of Swertia chirata leaf extracts

Type of extract	Organism	Plate count in control tube (ml)	Concentration of the Swertia chirata leaf extracts									
			Undiluted	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Alcoholic Extracts	E.Coli	600 x 10^6	0	0	0	0.84x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Staphylococcus aureus	600x10^6	0	1.72 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6

	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Aqueous Extracts	E.Coli	600 x 10^6	0	0	1.09 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Staphylococcus aureus	600 x 10^6	0	0	0	0	0.9 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6	600 x 10^6
	Aspergillus spore germination activity	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Table IX summary of antibacterial activity of different Plant extracts

S.No.	Name of plant	Against Escherichia coli		Against Staphylococcus aureus	
		Alcoholic extract end point dilution (MLC)	Aqueous extract end point dilution (MLC)	Alcoholic extract end point dilution (MLC)	Aqueous extract end point dilution (MLC)
1.	Moringa oleifera	1:2	1:2	1:32	1:4
2.	Withania somnifera	1:2	1:2	1:128	1:2
3.	Citrullus colocynthis	1:4	1:2	1:8	Nil
4.	Salvadra oleoids	1:2	Nil	1:64	1:2
5.	Swerita chirata	1:8	1:4	1:2	1:16

Table X :

Summary of antifungal activity of plant extract against Aspergillus species

Citrulluscolocynthis											
Name of plants	Control	-	-	-	-	-	-	-	-	-	-
		Alcoholic extract Concentration of the plant					Aqueous extract Concentration of the plant				
Salvadra oleoids		+	+	+		-	-	-	-	-	-
		extract					extract				
		Undiluted	1:2	1:4	1:8	1:16	Undiluted	1:2	1:4	1:8	1:16
Moringa oleifera	Anti Aspergillus	-	-	-	-	-	-	-	-	-	-
Withania somnifera		-	-	-	-	-	-	-	-	-	-

TABLE NO. XI : MIC of aqueous plant extracts by turbidity method

S.No.	Name of Plants	Control	MIC against E. Coli Concentration plant extract						MIC against Staphylococcus aureus Concentration plant extract					
			0	1:2	1:4	1:8	1:16	1:32	0	1:2	1:4	1:8	1:16	1:32
1	Moringa Oleifea	Mcfarland reading 2 approximated 600 x 10^6 number of bacteria	-	-	1	1	2	2	-	1	1	1	2	2
2	Withania somnifera		-	-	1	1	2	2	-	-	1	1	2	2
3	Citrullus Colocynthis		-	-	1	1	2	2	-	1	2	2	2	2
4	Salvadora Oleoide		-	1	2	2	2	2	-	-	1	2	2	2

5	Swertia Chirata		-	-	-	1	2	2	-	-	-	-	1	1

1 = 300 x 10^6 2 = 600 x 10^6 - = Not measured

Table No. XII

Comparison of MIC and MLC of aqueous plant extracts againsts test bacteria

S.No.	*Name of Plant*	**Against Escherichia Coli**		**Against Staphylococcus aureus**	
		MIC	**MLC**	**MIC**	**MLC**
1	Moringa Oleifera	1:8	1:2	1:8	1:4
2	Withania somnifera	1:8	1:2	1:8	1:2
3	Citrullus	1:8	1:2	1:2	NIL

	colocynthis				
4	Salvadora oleoides	1:2	NIL	1:4	1:2
5	Swertia chirata	1:8	1:4	1:32	1:16

DISCUSSION

The present investigation was carried out to determine the antimicrobial activity of some of the plants commonly found in desert ecosystem of the Bikaner area. These plants components and derivatives are known to be used in the treatment of infectious and non infectious diseases by traditional practitioners and also as biostimulants and nervine tonics.

Microorganisms are important component of health related illnesses or goodness as they affected the skin and many internal organs in men and all animals. It is known that normal micro biota synthesize many nutrients essential for health. Metabolites used by normal microorganisms also inhibit growth and lodgment of pathogenic microorganism. Determination of antimicrobial activity of selected plants, if any, becomes important in the light of influence on normal micro biota as well as in inhibition of pathogenic organisms.

For the determination of antimicrobial activity of plants aqueous and alcoholic extractions were attempted using established procedures. Test bacterial organisms were isolated from cattle mastitic milk sample and calf diarrhoeic faecal samples with the purpose of testing one gram positive i.e. Staphylococcus aureus and one gram negative i.e. Escherichia coli organisms which are potentially pathogenic i.e. Isolated from mastitic milk and diarrhoeic faecal samples, respectively. The test isolate Staphylococcus aureus exhibited morphological and biochemical characteristics (Cown and steel, 1975) typical for gram positive organism as mentioned in Table I.

Similarly the Escherichia coli isolate displayed characteristic features of the species in Table II. These test organisms were also studied for their antibiotic sensitivity resistance pattern in Table III. Since antibiotics are commonly used in the treatment of much illness, bacterial isolates seem to have developed resistance to some of them. Staphylococcus aureus was found resistant to Amoxycillin, Ampicillin, Ciprofloxacin, Doxycycline, Methicillin, Penicillin, Sulfadiazine and E. Coli was found to be resistant to be Amoxycillin, Bacitracin, Ciprofloxacin, Penicillin, Sulfadiazine, Vancomycin. These results are in conformatory to earlier findings of Mittal (1997) and Chatterji (2004) who found similar resistance pattern of Staphylococcus aureus and E. Coli isolates, respectively.

Extraction of plant phytochemicals

For the extraction of phytochemicals, water and organic solvents are routinely used. Water soluble polysaccharides, polypeptide, including fabatine and lectins of plant origin act as an inhibitors of microbial parthogens (Zhang and Lewis 1997).

Nearly all of the identified components from plants acting against pathogens are aromatic or saturated organic compounds. They are more efficiently extracted using organic solvents such as methanol, ethanol etc. In the present investigation boiling water and ethanol were used as solvents for aqueous and alcoholic extraction of phytochemicals. A concentration of 3:1 (W/V) and 5:1 (W/V) could be achieved in aqueous and alcoholic extraction from dried plant parts, respectively. Natural product chemists have recommended several organic solvents for the extraction of phytochemicals which include acetone, methanol and ethanol.

Among these acetone is considered superior (Eloff, 1998). Considering the biohazard of traces left and removal of the solvent from the function, ethanol was preffered in the present investigation.

Following removal of traces of ethanol, alcoholic extract was left as dark coloured, pasty material. The initial dilutions of alcoholic extract were found inconvenient and inaccurate in determining the turbidity. As such alcoholic extracts could not be used to determine MIC in turbidometric methods; however, aqueous extracts could be used for this purpose.

Antibacterial and antifungal activity of plant extracts

I. Antibacterial and antifungal activity of Moringa oleifera leaf extracts

Moringa oleifera popularly known as Sanjana is considered to have very important strong antiarthritic activity. In the present study, its alcoholic and aqueous extracts were found to possess antibacterial activity both against E. coli as well as Staphylococcus aureus in Table IV. Alcoholic extracts of Moringa oleifera had MLC against E. coli as 1:4 while against Staphylococcus aureus as 1:32. Its aqueous extracts showed MLC against E. coli in 1:2 dilution and against Staphylococcus aureus in 1:4 dilution. In turbidity measurement methods aqueous extract was found to inhibit the growth of both E. coli and Staphylococcus aureus up to dilution of 1:8 in Table XI. No fungal activity could be recorded in anti Aspergillus spore germination assay by aqueous and alcoholic extracts of Moringa oleifera. These results are in confirmation to the studies of Duero (1984), who found similar activity of Moringa oleifera aqueous and alcoholic extracts against E. coli and Staphylococcus aureus.

Gamila et al. (2004), reported fluctuation in antibacterial activity of Moringa oleifera against Staphylococcus aureus and E. coli. Caceres et al (1991), also found antimicrobial activity of Moringa oleifera but this activity was found to be lost when temperature was more than 56 ^{o}C.

No such inhibitory activity was found in our results as our extraction methods involved temperature of 100 ^{o}C in aqueous extraction. Our results regarding no antifungal activity of Moringa oleifera are in confirmatory to Caceres et al (1991), who also did not observed any antifungal activity of Moringa oleifera. Our results are not in agreement with Emeruwa (1991) and Singh et. al (2003) who reported inhibitory activity of Moringa oleifera against Candida albicans, Penicillum, and Tricophyton species. It could have been due to the use of roots and seeds of plant for the preparation of extract which was not considered in our study.

II. Antibacterial and antifungal activity of Withania somnifera leaf extracts

The preparation of Withania somnifera (popularly known as Aswagandha), are widely used as an antiinflammatory, antiseptic, antitussive, anti anxiety and as overall rejuvenative. Its medicinal properties are based on certain steroidal alkaloids and steroidal lectons called withanolides. In our study it is evident from the Table V that Withania somnifera leaf extracts displayed potent antibacterial activity against Staphylococcus aureus with MLC 1: 128. However, only undiluted extracts showed activity against E. Coli. Its aqueous extracts showed moderated antibacterial activity with MLC against Staphylococcus aureus 1:4 and against E. coli 1: 2.

In turbidometric methods aqueous extracts of Withania somenifera showed inhibitory activity against E. coli up to dilution 1 : 8 and against Staphylococcus aureus up to dilution 1 : 16. Both alcoholic and aqueous extracts did not show any antifungal activity.

These results are in confirmation to earlier findings of Jaffer et al (1998), who found significant antibacterial activity of Withania somnifera against gram positive bacteria and no any antimicrobial activity against gram negative bacteria.

Similarly Kazmi et al. (1991) and Ramadan et al. (1994) also found Withania somnifera root extracts having strong inhibitory activity against Staphylococcus aureus, Sterptococcus and Corynebacterium. Contrary to these findings Arora et al. (2004) and Owais et al. (2005) found antibacterial activity of Withania somnifera against E. coli and Salmonella typhimurium.

It could have been due to the fact that they used methanol and ether extracts of leaves and roots of Withania somnifera but in our case ethanol extract was used.

Mothana and Lindequist (2005) and Arora et al. (2004) have also reported that only methanol and ether extracts of Withania somnifera have antibacterial activity against gram negative organisms. Our results regarding no antifungal activity of Withania somnifera extracts are not in conformatory to the findings of Juffer (1988) and Kazmi et al. (1991) who found Withania somnifera inhibitory to the Trycophyton, Microsporium, Aspergillus and Candida species.

From all these studies it can be concluded that Withania somnifera has important antibacterial activity against gram positive bacteria especially Staphylococcus aureus and little or moderate activity against gram negative bacteria. The mechanism of its antibacterial activity needs further study.

III. Antibacterial and antifungal activity of Citrullus colocynthis fruits extracts

Citrullus colocynthis is popularly known as tumba or bitter apple and is traditionally used as drastic purgatives in desert rural areas. In present study its alcoholic and aqueous extracts in Table VI were found to possess antibacterial activity against in Staphylococcus aureus and E. coli.

Alcoholic extracts of fruit of Citrullus colocynthis showed MLC against E. coli as 1 : 4 dilution while against Staphylococcus aureus as 1 : 8 dilution. Its aqueous extracts had MLC against E. coli as 1 : 2 dilution but there was no lethal activity against Staphylococcus aureus. In turbidometric methods the aqueous extract was found against Staphylococcus aureus (in Table XI). No antifungal activity could be recorded against Aspergillus species. These results are in conformatory to the earlier findings of Memon et al (2003) who found antibacterial activity of ethanolic extracts of fruits, leaves and roots from Citrullus colocynthis against both gram positive and gram negative bacteria. In our results the antibacterial activity of ethanolic extracts of Citrullus colocynthis against E. coli is contrary to earlier findings of Menon et al. (2003) who found no antibacterial activity of ethanolic extracts of Citrullus colocynthis against E. coli and Psedomonas aeruginosa.

IV. Antibacterial and antifungal activity of Salvadora oleoides leaf extracts

Salvadora oleoids was studied for the determination of their antimicrobial activity as showned in Table VII. This species was found to possess antibacterial as well as antifungal activities.

The alcoholic extracts of Salvadora oleoides displayed limited activity against E. coli with MLC 1 : 2 dilurion and strong activity against Staphylococcus aureus with MLC 1 : 64 dilution. Aqueous extracts, however showed no activity against E. coli and limited activity against Staphylococcus aureus with MLC 1 : 2 dilution. In turbidity measurement methods (Table XI) the aqueous extract was found to inhibit the growth of E. coli up to dilution 1 : 2 and Staphylococcus aureus up to dilution 1 : 4.

Alcoholic extracts was found to found to possess antifungal activity against Aspergillus species in spore germination assay up to dilution of 1: 4. Aqueous extracts however failed to show this activity.

Salvadora stems are commonly used as tooth cleaner in Africans countries as well as in desert area of Rajasthan and thus the popular name of this plant Toothbrush tree. It is known to have strong anti Staphylococcus aureus activity which was also found in our results. In our results alcoholic extracts of Salvadora oleoides was strongly active against Staphylococcus aureus and limited activity against E. coli.

V. Antibacterial and antifungal activity of Swertia chirata leaves extracts

Common name of Swertia chirata is Chirayata which is found in greater India. Alcoholic extracts of leaves showed antibacterial activity against Staphylococcus aureus with MLC of 1 : 16 dilution and antibacterial activity was recorded against E. coli with MLC 1 : 4 in Table VIII. Alcoholic extracts showed better antibacterial activity against E. coli and somewhat activity against Staphylococcus aureus. In turbidity measurement methods (Table XI) the aqueous extract was found to inhibit the growth of E. coli up to dilution 1 : 8 and against S. aureus up to dilution 1: 32.

CONCLUSIONS

CONCLUSIONS

From the above results it could be concluded that:

I. Alcoholic extract preparations of all the plants tested against different microorganisms showed higher antibacterial activity than the aqueous extract preparations.

II. The antibacterial activity of alcoholic extract preparations of all the plants was more effective against Staphylococcus aureus than against E.coli.

III. Withania somnifera had the highest and strongest anti Staphylococcus aureus activity followed by Salvadora oleoides. and Moringa oleifera .

IV. Citrullus colocynthis extract preparation showed highest activity against E. coli and all other plant extracts preparation mainly aqueous extracts, demonstrated moderate anti E. Coli activity

V. From all of the plant extract preparation only Salvadora oleoides plants alcoholic extracts showed antifungal (anti aspergillus) activity.

SUMMARY

SUMMARY

The present investigation was planed to determine antibacterial and antifungal activity of plants commonly found in desert ecosystem. The plants selected for this purpose are Moringa oleifera, Withania somnifera, Citrullus colocynthis, Salvadora oleoides, and Crotolaria burhia.

For determination of antibacterial activity of these plants, pathogenic Staphylococcus aureus was isolated from cattle mastitic milk and E. coli was isolated from faeces of calf diarrhoea. The test organisms were analysed for their antibiotic sensitivity pattern. Staphylococcus aureus was found sensitive to antibiotics Bacitracin, Chloramphenicol, Ciprofloxacin, Gentamycin, Kanamycin, Neomycin, Vancomycin and it was found resistant to Amoxycillin, Ampicillin, Ciprofloxacin, Doxycycline, Methicillin, Penicillin, and Sulfadiazine. E. coli was found to sensitive to antibiotics Amoxycillin, Chloramphenicol, Ciprofloxacin, Doxycycline, Gentamycin, Kanamycin, Methicillin, Neomycin and it was found resistant to Amoxycillin, Bacitracin, Ciprofloxacin, Penicillin, Sulfadiazine, Vancomycin. To determine antifungal activity Aspergillus Fumigatus was isolated from sheep nasal swab and identified on the basis of its morphological characteristic.

Alcoholic and aqueous extraction methods were employed for separation of phytochemicals from different plant components.Antibacterial activity of these plant extracts could be determined in terms of minimum lethal activity (MLC) in plate count assay and minimum inhibitory concentration (MIC) in turbidometric assay of test bacteria. Antifungal activity was determined by inhibition of spore germination of test fungi. The results indicated that alcoholic extracts of all the plants tested displayed higher antibacterial and antifungal activity than the aqueous extracts.

The antibacterial activity of alcoholic extracts of all the plants tested was more effective against Staphylococcus aureus than the aqueous extracts.

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CERTIFICATE

This is to certify that **Mr. Harit Kumar Rawal** was involved in the project entitled "**Invitro Antimicrobial Activity Of Plant Extracts By Turbidity Method**" conducted at College Of Veterinary & Animal Science, Department Of Veterinary Microbiology & Biotechnology, Bikaner and has submitted the project report on the same as per the requirement of **Dr.M.G.R MEDICAL UNIVERSITY OF TAMILNADU** for the study course M.Pharm. (Pharmacology) part –II, under the supervision of **Mr.R.Suresh** Professor,

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DECLARATION

As required by university regulation, I wish to state that this work embodied in this thesis titled "**Invitro Antimicrobial Activity Of Plant Extracts By Turbidity Method**" forms my own contribution to the research work carried out under the guidance of **Mr. R. Suresh & Dr. B. N. Shringi**. This work has not been submitted for any other degree of this or any other university. Whenever references have been made to previous work of others, it has been clearly indicated as such and included in the bibliography.

Signature of the Candidate

Mr. Harit Kumar Rawal